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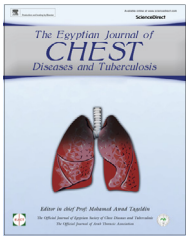

ORIGINAL ARTICLE

Association between HCV induced mixed cryoglobulinemia and pulmonary affection: The role of TNF-alpha in the pathogenesis of pulmonary changes

AbdelBaset M. Saleh [a,*], Hatem Elalfy [b], Mona M. Arafa [c], Nashwa Abousamra [c], Adel El-Badrawy [d], Mie A. Mohamed [e], Enaase A.M.E. Barakat [f], Basem S. El Deek [g,h]

[a] *Thoracic Medicine Department, Faculty of Medicine, Mansoura University, Egypt*
[b] *Tropical Medicine Department, Faculty of Medicine, Mansoura University, Egypt*
[c] *Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt*
[d] *Radiology Department, Faculty of Medicine, Mansoura University, Egypt*
[e] *Pathology Department, Faculty of Medicine, Mansoura University, Egypt*
[f] *Internal Medicine Departement, Faculty of Medicine, Mansoura University, Egypt*
[g] *Community Medicine Department, King Abdulaziz University, Saudi Arabia*
[h] *Community Medicine Department, Mansoura University, Egypt*

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KEYWORDS

Chronic hepatitis C virus;
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Abstract *Background and aim of the work:* Chronic hepatitis C virus (HCV) infection is associated with both pulmonary involvement and cryoglobulinemia. Therefore, this study was designed to investigate the relationship between pulmonary involvement and mixed cryoglobulinemia in chronic HCV infected patients and to investigate the role of TNF-alpha in the pathogenesis of pulmonary changes.

Subjects and methods: After hospital ethics committee approval and formal patient consent were obtained, 100 patients with compensated hepatitis C virus infection as confirmed by PCR were recruited in this cross sectional study. Their demographic and laboratory data, abdominal ultrasound findings, pulmonary function tests (spirometry), arterial blood gas (ABG) parameters, TNF-alpha levels, and data from high-resolution chest CT were collected and analyzed using SPSS version 16, and a serum cryoglobulin assay was performed in all of the studied patients.

* Corresponding author. Tel.: +966 595621176; fax: 20 50 2260138.
E-mail addresses: baset.m.saleh@gmail.com, im4.med@sghgroup.net (AbdelBaset M. Saleh).

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Results: The prevalence of mixed cryoglobulinemia was 61.7% in the studied HCV patients. Pulmonary symptoms were observed in more than half of these patients. The most common complaint among the symptomatic patients was dyspnea (51.7%), followed by cough (43.3%). Oxygen saturation (Spo_2 and Sao_2%), and FEV_1 and FVC levels, were significantly decreased in the cryoglobulin positive patients compared to the cryoglobulin negative patients. A statistically significant correlation was found between the presence of cryoglobulins and FEV_1 level, FVC level, serum albumin level, viremia level, thrombocytopenia and arterial blood gas parameters. No correlation was found between cryoglobulinemia and TNF-alpha level.

Conclusions: The results of this study suggest that pulmonary involvement is common in patients with chronic HCV infection and mixed cryoglobulinemia. Cryoglobulinemia may lead to pulmonary involvement through vascular and interstitial deposition of cryoglobulins, which results in impaired gas exchange and airway affection.

Introduction

Chronic hepatitis C virus (HCV) infection is a major public health problem worldwide, and more than 170 million people are chronically infected with HCV (approximately 3% of the world's population) [1]. In Egypt, the situation is quite worse because the overall anti-HCV antibody prevalence is 14.7% [2].

HCV predominantly affects the liver; however, it can also produce a number of extra hepatic manifestations, such as mixed cryoglobulinemia (MC), which is the most common and severe extra hepatic manifestation. HCV is a systemic vasculitis involving the small- and medium-sized arteries and veins. MC is characterized by the deposition of immune complexes containing mainly rheumatoid factor, IgG, HCV RNA, and complement on endothelial surfaces, causing vascular inflammation through poorly understood mechanisms [3]. Moreover, many inflammatory diseases are known to be associated with the overproduction of cytokines such as TNF-alpha. TNF-alpha performs several proinflammatory functions including the promotion of leukocyte–endothelium interactions and the activation of the arachidonic acid pathway [4].

The prevalence of mixed cryoglobulinemia is related to the endemic presence of HCV infection, and its prevalence varies widely between countries, ranging from 10–70%; this geographical heterogeneity may be due to population selection and time lead bias [1]. Chronic HCV infected cryoglobulinemic patients have an apparent duration of HCV infection that is almost twice as long as that in chronic HCV infected non cryoglobulinemic patients, thereby suggesting that MC is associated with an increased duration of HCV infection [5].

Many MC patients are asymptomatic. MC is diagnosed when a patient has a typical organ involvement (mainly skin, kidney, or peripheral nerves) in addition to circulating cryoglobulins. Cutaneous purpura is the most common manifestation of cryoglobulinemic vasculitis. The most frequently affected internal organs are the peripheral nerves, kidneys, lungs and joints [6].

Pulmonary involvement in MC is usually mild, although severe pulmonary complications including diffuse alveolar damage, organizing pneumonia and hemoptysis have been reported [7]. However, a few studies have demonstrated pulmonary involvement in chronic HCV infection with and without cryoglobulinemia; the prevalence of pulmonary involvement in MC is not well understood.

Therefore, this study was designed to investigate the relationship between pulmonary involvement and mixed cryoglobulinemia in chronic HCV infected patients and to investigate the role of TNF-alpha in the pathogenesis of pulmonary changes. Understanding the epidemiology of the relationship between pulmonary involvement and mixed
cryoglobulinemia in chronic HCV infected patients and the pathogenesis of pulmonary changes will provide novel insights into better management, especially in the present era of liver transplantation, in which the presence of advanced pulmonary affection may be considered as a major hurdle in the treatment of chronic HCV infected patients.

Materials and methods

Study design

This was a cross sectional study including 100 patients with compensated HCV infection (69 males and 31 females) with mean age 54.63 years.

Sample size and power of the study

The sample size was calculated using the medcalc program available at www.medcalc.be on 21-2-2011. The confidence level for our study was 95% with an alpha error of 0.05. The power of this study was set at 80% with a beta error of 20%. The maximum prevalence of mixed cryoglobulinemia was considered to be 60%. The minimal prevalence of mixed cryoglobulinemia was considered to be 50%. The estimated sample size was 195 patients. Two hundred forty patients with chronic HCV infection were screened for study eligibility, and 100 patients were recruited in the study according to the inclusion and exclusion criteria.

This study was performed at the Tropical Medicine Department, in collaboration with the Thoracic Medicine, Clinical Pathology, Pathology and Radiology Departments, Mansoura University Hospital, between May 2010 and December 2011. One hundred patients with chronic HCV

infection as confirmed by PCR and abnormal liver function tests were included.

Patient selection

This study included a convenient sample of patients such as those attending the inpatient and outpatient clinics of the Tropical Medicine Unit, and all of the patients provided written informed consent. The Institutional Review Board (IRB) of our Faculty of Medicine approved the study.

Inclusion criteria for patient selection were as follows:

1. Adults
2. Any gender
3. Egyptian nationality
4. HCV infection documented by PCR
5. Abnormal liver function tests

- The patient group was further subdivided into two subgroups according to the presence or absence of cryoglobulinemia (mean age of HCV infected cryonegative patients was 45.5 ± 5.89 years and that of HCV infected cryopositive patients was 47.8 ± 7.98 years).

Exclusion criteria were as follows:

- Patients with decompensated liver disease (i.e. ascites, encephalopathy and coagulopathy which are the signs of liver cell failure).
- Patients with previous lung disease (prior to the diagnosis of HCV).
- Smokers.
- Patients with previous systemic diseases (such as renal failure, congestive heart failure and connective tissue disorders).
- Patients with a current history or past history of antiviral treatment for hepatitis C.
- Patients with autoimmune liver diseases or any other liver disease.

All of the patients were subjected to the following:

Thorough history taking and clinical examination, serologic assays and PCR for HCV and HBs Ag, liver function tests and alpha fetoprotein (AFP) test for screening of hepato-cellular carcinoma (HCC) were performed. Abdominal ultrasound and triphasic CT abdomen were also performed. To assess the severity of liver disease, liver biopsy was performed according to Ishak et al. [8] scoring system.

In addition, all of the patients were subjected to arterial blood gas (ABG) analysis, pulmonary function tests (spirometry) and chest high resolution CT scan (HRCT).

Arterial blood gas analysis

ABG samples were obtained by a percutaneous radial artery puncture in stable seated patients while breathing room air. Then, the pH, partial pressure of oxygen in arterial blood (PaO_2), partial pressure of carbon dioxide in arterial blood ($PaCO_2$) and other parameters were measured with a standard blood gas analyzer (Eschweiler compact PGA, serial No. P2084, GERMANY).

Pulmonary function tests

Forced expiratory volume in first second (FEV_1), forced vital capacity (FVC), forced mid-expiratory flow rate (FEF25-75), and FVC/FEV_1% were measured and recorded using a spirometer by a trained, experienced chest physician. Spirometry was performed in accordance with American Thoracic Society criteria. Three technically acceptable measurements were obtained for each patient, and the highest value was included in the analyses (Spirolab 2, serial No.A23–050.09978MIR, ITALY)

Cryoglobulin testing

Cryoglobulins were detected by the Winfield method [9]. Twenty milliliters of venous blood was obtained from each patient in a pre-warmed (37 °C) syringe. The blood was allowed to clot at 37 °C, and the serum was separated by centrifugation. The supernatant was incubated at 4 °C for 8 d and was examined daily for the cryoprecipitate.

Assessment of tumor necrosis factor-alpha (TNF-alpha) levels

The evaluation of serum TNF-alpha levels was performed using a commercially available enzyme-linked immunosorbent assay, human TNF-alpha ELISA kit (RayBio, GA, USA), according to the manufacturer's instructions.

Liver biopsy. Liver biopsy specimens were fixed in 10% formalin, and they were stained with hematoxylin, eosin and Masson's trichrome. The specimens with more than six portal areas were used for examination. The severity of chronic liver disease (CLD) was estimated according to Ishak et al. [8] scoring system proposed in 1995 (fibrosis 0–6, and liver biopsy was suitable).

Statistical analysis

The statistical package for the Social Sciences (SPSS) version 16 was used for the statistical analysis. The qualitative data were presented in the form of numbers and percentages. The Chi-square test was used as a test of significance for the qualitative data, and Yates correction was used when the expected cell count was less than 5. The quantitative data were expressed as mean and standard deviation. Independent sample t test was used to compare the quantitative data between two groups. Pearson correlation was used to study the relationship between variables. Statistical significance was considered at a p value less than 0.05.

Results

This study showed that the prevalence of cryoglobulinemia was 61.7% in the studied HCV patients.

Table 1 summarizes the patients' clinical data. Pulmonary symptoms were found in less than half of the patients; however, there was no difference in the pulmonary symptoms between the cryoglobuin positive patients and the cryoglobulin negative patients. Dyspnea and cough were the most common complaints among the symptomatic patients. Some patients had more than one pulmonary symptom. A statistically significant difference was found in the extra pulmonary manifestations, such as vasculitis, rash, renal impairment,

Table 1 Clinical data of the studied compensated HCV patients according to the presence or absence of cryoglobulins.

	Cryoglobulin positive patients (n = 52) N (%)	Cryoglobulin negative patients (n = 48) N (%)	Test of significance
Renal involvement	4 (8.3)	–	$P = 0.034$
DM	28 (58.3)	8 (15.4)	$P < 0.001$
Arthralgia	16 (33.3)	4 (7.7)	$P = 0.002$
Elevated purpura (vasculitis rash)	24 (50)	8 (15.4)	$P < 0.001$
Lichen planus	4 (8.3)	–	$P = 0.034$
Lower limb pigmentation	20 (41.7)	12 (23.1)	$P = 0.046$
Cough	20 (41.7)	16 (30.8)	$P = 0.177$
Dyspnea	20 (41.7)	20 (38.5)	$P = 0.45$

arthralgia, lichen planus and diabetes, between the cryoglobulin positive patients and the cryoglobulin negative patients.

Table 2 summarizes the lung function abnormalities (diagnosed by ABG analysis and spirometry) and laboratory findings of the HCV patients with and without cryoglobulinemia. The Spo2, Sao2%, FEV1 and FVC levels were significantly decreased in the cryoglobulin positive compensated HCV patients as compared to the cryoglobulin negative compensated HCV patients; however, the clinical data showed no difference between the groups in terms of age, sex and disease duration.

There was a significant increase in the serum albumin level and viremia level in the cryo-positive HCV patients, whereas liver enzymes (ALT and AST) were significantly elevated in the cryo-negative HCV patients.

However, the comparison of chest HRCT findings in both the groups showed no significant difference. The non-septal lines, pulmonary vascular congestion and septal lines were the most common chest HRCT findings, followed by ground glass attenuation. Some patients showed more than one abnormality on chest HRCT.

Table 3 shows that there was a statistically significant correlation between the presence of cryoglobulins and FEV_1 level, FVC level, serum albumin level, viremia level, thrombocytopenia and arterial blood gas parameters (PaO_2 and O_2 saturation). No significant correlation was found between the presence of cryoglobulins and pulmonary symptoms (such as, cough and wheezing) or extra-pulmonary findings (such as, arthralgia, purpura, lichen planus and lower limb vasculitis). Finally, as shown in Table 3, there were significant negative correlations between cryoglobulin positivity and ALT and AST levels; however, no correlation was found between cryoglobulin positivity and other liver function tests.

Hepatic fibrosis was evaluated using the Ishak score; it showed a significant correlation with pulmonary function parameters, including FEV_1/FVC, O_2 saturation and PaO_2 (P values 0.005, 0.026, respectively), whereas no significant correlation was found between fibrosis score and cryoglobulinemia (Data not shown).

Table 4 demonstrates the TNF-alpha levels in both the cryonegative patients and the cryopositive patients, which may indicate the underlying pathophysiological role of TNF-alpha in causing pulmonary changes.

The median TNF-alpha level in the cryopositive patients was higher than that in the cryonegative patients; however, this difference was not statistically significant.

Table 2 Clinical data, blood gas analysis, pulmonary function tests and laboratory tests of the studied compensated HCV patients according to the presence or absence of cryoglobulins.

	Cryoglobulin negative patients (n = 48)	Cryoglobulin positive patients (n = 52)	Test of significance
Age (Mean ± SD)	45.5 ± 5.89	47.8 ± 7.98	$P = 0.115$
Disease duration (years)	10.5 ± 3.23	11.3 ± 4.34	$P = 0.163$
Gender male/female	30/18	39/13	$P = 0.66$
ABG			
PaO_2 (mmHg)	130.5 ± 29.31	83.5 ± 36.1	$P < 0.001$
O_2 saturation	97.71 ± 1.63	92.7 ± 4.57	$P < 0.001$
pH	7.37 ± 0.148	7.39 ± 0.042	$P = 0.73$
$PaCO_2$ (mmHg)	35.66 ± 6.41	32.04 ± 8.08	$P = 0.25$
Pulmonary function tests			
FEV_1/FVC (%)	98.15 ± 12.72	104.81 ± 1.37	$P = 0.081$
FEV_1 (% predicted)	89.61 ± 11.45	79.16 ± 16.72	$P = 0.013$
FVC (% predicted)	88.53 ± 12.96	76.41 ± 21.86	$P = 0.020$
FEF25–75	97.84 ± 15.92	73.83 ± 0.83	$P = 0.003$
Laboratory tests			
Bilirubin (mg/dL)	0.98 ± 0.20	0.96 ± 0.32	$P = 0.96$
ALT (IU/dL)	78.58 ± 60.67	59 ± 15.61	$P = 0.027$
AST (IU/dL)	90.16 ± 72.43	65.38 ± 24.29	$P = 0.022$
S. Albumin (g/dL)	3.9 ± 0.64	4.15 ± 0.49	$P = 0.035$
HCV viral load (IU/ml)	222.33 ± 304.51	440.27 ± 337	$P < 0.001$

Table 3 Correlation between the presence of cryoglobulins, clinical and laboratory data, pulmonary function tests (PFT) and arterial blood gas parameters (ABG) in the compensated HCV patient group.

Clinical data	Presence of cryoglobulins	Laboratory data	Presence of cryoglobulins	PFT & ABG	Presence of cryoglobulins
Diabetes mellitus	−0.103	ALT	−0.226	PaO_2	−0.59
Arthralgia	0.115	AST	−0.242	O_2 saturation	−0.56
Elevated purpura	−0.107	Serum albumin	0.20		
Lichen planus	−0.189			FEV_1/FVC	−0.22
Lower limb pigmentation	0.104			FEV_1	0.389
Cough	0.05	HCV viral load	0.226	FVC	0.341
Wheezing	0.189	Thrombo-cytopenia	0.390	FEF25–75	0.248

Table 4 Serum TNF-alpha levels (pg/ml) in the studied compensated HCV patient groups.

	Cryoglobulin negative compensated HCV patient group ($n = 48$)	Cryoglobulin positive compensated HCV patient group ($n = 52$)	Control ($n = 72$)	P value
TNF-alpha	34.39 ± 8.29	39.89 ± 19.11	22.42 ± 6.19	<0.001

Cryoglobulin negative compensated HCV patient group versus Control ($P < 0.001$).
Cryoglobulin positive compensated HCV patient group versus Control ($P < 0.001$).

Discussion

This study showed that mixed cryoglobulinemia is prevalent in HCV patients (61.7%). Oxygen saturation (Spo_2 and Sao_2%), and FEV_1 and FVC levels were significantly decreased in the cryoglobulin positive patients than in the cryoglobulin negative patients. A statistically significant correlation was found between the presence of cryoglobulins and FEV_1 level, FVC level, serum albumin level, viremia level, thrombocytopenia and arterial blood gas parameters.

This study showed that the prevalence of mixed cryoglobulinemia was 61.7% in the studied HCV patients. This result is within the range of the previous estimates for the prevalence of MC in HCV infection, which varies widely from 10% to 70%. According to different studies, MC can be found in 19–50% of patients with HCV infection. At the same time, only a small fraction of these HCV infected patients (less than 15%) have symptomatic mixed cryoglobulinemia. However, the asymptomatic mixed cryoglobulinemic patients may develop CG-related symptoms during the course of the disease. Factors that seem to favor the development of MC are female gender, age, alcohol intake (> 50 g/d), advanced liver fibrosis and steatosis [10–12].

HCV is a well-known cause of liver fibrosis, and it could potentially provoke similar abnormalities in the lung, mainly because of its lymphotropism, which can induce chronic immune activation and inflammation [13]. Importantly, in this study, pulmonary symptoms were found in more than half of the patients. Dyspnea, which is the main symptom of pulmonary interstitial affection, was the most common complaint among the symptomatic patients (51.7%), followed by cough in 43.3% and expectoration in 1.7% of patients. Some patients had more than one pulmonary symptom. Our findings are in agreement with those of previous studies which reported that HCV infection was complicated by a number of extrahepatic manifestations, and it is associated with both the obstructive and restrictive lung diseases [14,15]. However, for some accompanying disorders, such as mixed cryoglobulinemia, the pathogenetic role of the HCV is substantiated by the epidemiologic and experimental data [16,17].

Importantly, a significant increase was found in the serum albumin level (thereby increasing blood viscosity) and HCV viremia levels in the cryo-positive patients, which suggest an increased deposition of cryoglobulins in the pulmonary interstitial tissue and in the airway. These findings are manifested by impaired gas exchange and obstructive ventilatory defect. In addition, these findings are in agreement with those of a previous study, which reported that the most common clinical features of hepatitis C associated with mixed cryoglobulinemia (MC + HCV) are correlated with those of vasculitis affecting various organs and sometimes with those of an increased viscosity of the plasma [18]. Many MC + HCV patients experience symptoms, such as fatigue, dyspnea and reduced physical activity. However, in many patients, these symptoms are not proportional to the liver involvement [18].

Some evidence suggests that alveolitis and HCV infection may not be coincidental findings [19,20]. In addition, the prevalence of serum antibodies against HCV (determined using first-generation enzyme-linked immunosorbent assay) has been reported to be higher in patients with idiopathic pulmonary fibrosis (IPF) than in controls, although a subsequent study using similar but improved techniques refuted this finding [21,22]. Further evidence of an association between HCV and interstitial lung involvement was provided by Ferri et al. [23]. Our study reported a significant two fold increase in the HCV viremia levels in the cryo-positive patients compared with the cryo-negative patients.

Mixed cryoglobulinemia is a well-described complication of HCV infection [24]. Fibrotic lung disease is likely to result from an inciting injurious event within the lung. However, the sequence of events and mechanisms of fibrotic lung disease are not understood. An emerging hypothesis is that occult infections may play a pathogenetic role as

cofactors in the development of pulmonary fibrosis, and this hypothesis is based on the assumption that an inflammatory agent (such as a virus) disrupts the physiologic healing response, thereby making the lung highly susceptible to injurious triggers [25]. Chronic HCV infection may contribute to the immune responses that modulate the pathogenic processes underlying pulmonary disorders; therefore, chronic HCV infection may produce a wide spectrum of clinical presentations. The inflammatory cytokines are the potential candidates that play a role in these immune responses [26].

In this study, both cryoglobulinemic patients and non cryoglobulinemic patients showed significantly higher mean TNF-alpha levels than those in controls; however, no significant difference in the mean TNF-alpha levels was demonstrated between cryoglobulinemic and non cryoglobulinemic patients. On the other hand, another study reported significantly higher serum TNF-alpha levels in the HCV–MC patients than in HCV+ patients or in controls [18]. In addition, other studies reported elevated serum levels of B cell-activating factor (BAFF), a TNF-alpha family member, which is required for B cell survival in HCV–MC, although the underlying mechanism remains unclear [27–30].

Thus, our study confirmed the finding of a high serum TNF-alpha level in HCV +ve patients with or without cryoglobulinemia, as was previously demonstrated in other studies of HCV +ve patients [31–33]. The increase in the TNF-alpha level in MC + HCV patients was unlikely to be due to a more aggressive liver disease; in fact, in our study, no correlation was found between the TNF-alpha levels and ALT levels, or between the TNF-alpha levels and the degree of liver inflammation. This result was in accordance with that of a previous study [34]. Other studies have shown an increased production of TNF-alpha by lymphocytes in MC + HCV patients [35,36], thereby suggesting that the increase in the TNF-alpha level may be due to the activation of lymphoid cells.

However, the severity of pulmonary involvement may or may not parallel the liver impairment; thus, patient management and prediction of disease outcome can be subject to marked variability. Therefore, early referral of MC + HCV patients who complain of any pulmonary symptoms to a specialized medical team in this field (pulmonologists and hepatologists) is strongly recommended.

Our study has two limitations. First, the study population may not be a representative of the general population in Egypt. Therefore, this study may have been affected by the selection bias. Second, genotyping of HCV and cryoglobulin quantitation were not performed because there was not enough financial aid to cover these expenses in the studied HCV cases.

Conclusion

Cryoglobulinemia is a laboratory finding, and it is not a clinical presentation. Cryoglobulinemia may have an impact on both gas exchange and airway parameters. Understanding the respiratory effects of HCV, which is considered to be a major health problem in Egypt, might improve our approach in the treatment of respiratory problems complicating HCV. Future studies should focus on evaluating the pathophysiological mechanisms underlying the relationship between mixed cryglobulinemia and pulmonary affection in chronic HCV patients.

Conflict of interest

None.

References

[1] E.D. Charles, L.B. Dustin, Hepatitis C virus-induced cryoglobulinemia, Kidney Int. 76 (8) (2009) 818–824.
[2] El.-Zanaty. Fatma, Ann. Way, Egypt Demographic and Health Survey 2008, Ministry of Health, El-Zanaty and Associates, and MacroInternational, Cairo, Egypt, 2009, http://www2.hawaii.edu/≈dewolfe/Epidemic.html.
[3] C. Ferri, Mixed cryoglobulinemia, Orphanet J. Rare Dis. 3 (2008) 25.
[4] C.A. Dinarello, The biological properties of interleukin 1, Eur. Cytokine Netw. 5 (1994) 517–531.
[5] F. Lunel, L. Musset, P. Cacoub, Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage, Gastroenterology 106 (1994) 1291–1300.
[6] M. Ramos-Casals, J.H. Stone, M.C. Cid, X. Bosch, The cryoglobulinaemias, Lancet 379 (9813) (2012) 348–360.
[7] J. Moorman, M. Saad, S. Kosseifi, G. Krishnaswamy, Hepatitis C virus and the lung: implications for therapy, Chest 128 (2005) 2882–2892.
[8] K. Ishak, A. Baptista, L. Bianchi, Histological grading and staging of chronic hepatitis, J. Hepatol. 22 (1995) 696–699.
[9] J.B. Winfield, D. Koffler, H.G. Kunkel, Specific concentration of polynucleotide immune complexes in the cryoprecipitates of patients with systemic lupus erythematosus, J. Clin. Invest. 56 (1975) 563–570.
[10] F. Lunel, L. Musset, P. Cacoub, L. Franguel, P. Cresta, M. Perri, et al, Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage, Gastroenterology 106 (1994) 1291–1300.
[11] D. Saadoun, T. Asselah, M. Resche-Rigon, P. Bedossa, D. Valla, Cryoglobulinemia is associated with steatosis and fibrosis in chronic hepatitis C, Hepatology 43 (2006) 133–1345.
[12] V.S. Wong, W. Egner, T. Elsey, D. Brown, G.J. Alexander, Incidence, character and clinical relevance of mixed cryoglobulinaemia in patients with chronic hepatitis C virus infection, Clin. Exp. Immunol. 104 (1996) 25–31.
[13] A.L. Zignego, C. Ferri, M. Monti, Hepatitis C virus as a lymphotropic agent evidences and pathogenetic implications, Clin. Exp. Rheumatol. 13 (1) (1995) 33–37.
[14] G.M. Lauer, B.D. Walker, Hepatitis C virus infection, N. Engl. J. Med. 345 (2001) 41–52.
[15] H. Kanazawa, K. Hirata, J. Yoshikawa, Accelerated decline of lung function in COPD patients with chronic hepatitis C virus infection: a preliminary study based on small numbers of patients, Chest 123 (2003) 596–599.
[16] R. Misiani, P. Bellavita, D. Fenili, Hepatitis C virus infection in patients with essential mixed cryoglobulinemia, Ann. Intern. Med. 117 (1992) 573–577.
[17] V. Agnello, R.T. Chung, L.M. Kaplan, A role for hepatitis C virus infection in type II cryoglobulinemia, N. Engl. J. Med. 327 (1992) 1490–1495.
[18] A. Antonelli, C. Ferri, S.M. Ferrari, E. Ghiri, S. Marchi, M. Colaci, F. Bruschi, P. Fallahi, High interleukin-6 and tumor necrosis factor-alpha serum levels in hepatitis C infection associated or not with mixed cryoglobulinemia, Clin. Rheumatol. 28 (10) (2009) 1179–1185.
[19] R. Idilman, H. Cetinkaya, I. Savas, Bronchoalveolar lavage fluid analysis in individuals with chronic hepatitis C, J. Med. Virol. 66 (2002) 34–39.
[20] K. Kubo, S. Yamaguchi, K. Fujimoto, Bronchoalveolar lavage fluid findings in patients with chronic hepatitis C virus infection, Thorax 51 (1996) 312–314.

[21] W.L. Irving, S. Day, I.D. Johnston, Idiopathic pulmonary fibrosis and hepatitis C virus infection, Am. Rev. Respir. Dis. 148 (1993) 1683–1684.
[22] T. Ueda, K. Ohta, N. Suzuki, Idiopathic pulmonary fibrosis and high prevalence of serum antibodies to hepatitis C virus, Am. Rev. Respir. Dis. 146 (1992) 266–268.
[23] C. Ferri, L. La Civita, P. Fazzi, Interstitial lung fibrosis and rheumatic disorders in patients with hepatitis C virus infection, Br. J. Rheumatol. 36 (1997) 360–365.
[24] V. Agnello, F.G. De Rosa, Extrahepatic disease manifestations of HCV infection: some current issues, J. Hepatol. 40 (2004) 341–352.
[25] P. Spagnolo, S. Zeuzem, L. Richeldi1, R.M. du Bois, The complex interrelationships between chronic lung and liver disease: a review, J. Viral Hepatitis 17 (2010) 381–390.
[26] H. Kanazawa, Relationship between hepatitis C virus infection and pulmonary disorders: potential mechanisms of interaction, Expert. Rev. Clin. Immunol. 2 (5) (2006) 801–810.
[27] D.A. Landau, M. Rosenzwajg, D. Saadoun, The B lymphocyte stimulator receptor-ligand system in hepatitis C virus-induced B cell clonal disorders, Ann. Rheum. Dis. 68 (2009) 337–344.
[28] D. Sene, N. Limal, P. Ghillani-Dalbin, Hepatitis C virus-associated B-cell proliferation – the role of serum B lymphocyte stimulator (BLyS/BAFF), Rheumatology (Oxford) 46 (2007) 65–69.
[29] E. Toubi, S. Gordon, A. Kessel, Elevated serum B-Lymphocyte activating factor (BAFF) in chronic hepatitis C virus infection: association with autoimmunity, J. Autoimmun. 27 (2006) 34–139.
[30] M. Fabris, L. Quartuccio, S. Sacco, B-Lymphocyte stimulator (BLyS) up-regulation in mixed cryoglobulinaemia syndrome and hepatitis-C virus infection, Rheumatology (Oxford) 46 (2007) 37–43.
[31] A. Lecube, C. Hernández, J. Genescà, R. Simó, Proinflammatory cytokines, insulin resistance, and insulin secretion in chronic hepatitis C patients: A case-control study, Diabetes Care 29 (2006) 1096–1101.
[32] M.G. Neuman, J.P. Benhamou, P. Marcellin, D. Valla, I.M. Malkiewicz, G.G. Katz, C. Trepo, M. Bourliere, R.G. Cameron, L. Cohen, M. Morgan, H. Schmilovitz-Weiss, Z. Ben-Ari, Cytokine–chemokine and apoptotic signatures in patients with hepatitis C, Transl. Res. 149 (2007) 126–136.
[33] S.M. Riordan, N.A. Skinner, J. Kurtovic, S. Locarnini, C.J. McIver, R. Williams, K. Visvanathan, Toll-like receptor expression in chronic hepatitis C: correlation with pro-inflammatory cytokine levels and liver injury, Inflamm. Res. 55 (2006) 279–285.
[34] A. Antonelli, C. Ferri, P. Fallahi, S.M. Ferrari, M. Sebastiani, D. Ferrari, M. Giunti, S. Frascerra, S. Tolari, F. Franzoni, F. Galetta, S. Marchi, E. Ferrannini, High values of CXCL10 serum levels in mixed cryoglobulinemia associated with hepatitis C infection, Am. J. Gastroenterol. 103 (2008) 2488–2494.
[35] S. Loffreda, P. Muratori, L. Muratori, L. Mele, F.B. Bianchi, M. Lenzi, Enhanced monocyte Th1 cytokine production in HCVinfected cryoglobulinemic patients, J. Hepatol. 38 (2003) 230–236.
[36] D. Saadoun, O. Boyer, H. Trébeden-Nègre, N. Limal, V. Bon-Durand, M. Andreu, D. Klatzmann, J.C. Piette, P. Cacoub, Predominance of type 1 (Th1) cytokine production in the liver of patients with HCV-associated mixed cryoglobulinemia vasculitis, J. Hepatol. 41 (2004) 1031–1037.